Reference: *Biol. Bull.* **171**: 682–693. (December, 1986)

UPTAKE OF AMINO ACIDS BY *PAREURYTHOE CALIFORNICA*: SUBSTRATE INTERACTION MODIFIES NET INFLUX FROM THE ENVIRONMENT

STEVEN J. O'DELL AND GROVER C. STEPHENS

Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717

Abstract

Pareurythoe californica is capable of simultaneous net uptake of 18 amino acids, each present at an initial concentration of 200 n*M*. Rates of uptake are comparable for all amino acids tested. Kinetics of uptake are well described by the Michaelis-Menten equation. Neither bacteria nor other epifauna play a significant role in the observed uptake.

The net entry of the amino acids tested was inhibited in the presence of equimolar concentrations of representatives of all major classes of amino acids (*i.e.*, polar and nonpolar neutral, dicarboxylic, polybasic) at concentrations comparable to those found in the environment. Thus, a mixture mimicking the composition and concentration of substrates normally present in the environment was used to obtain a more realistic estimate of entry rates under natural conditions. When this was done, the contribution of exogenous amino acids to the nitrogen needs (based on ammonia excretion) and the requirement for reduced carbon (based on oxygen consumption) of *Pareurythoe* ranged from 10% to 50%, depending on levels of substrate available in the environment.

Introduction

High performance liquid chromatography (HPLC) has been used to demonstrate net uptake of amino acids by bacteria-free marine organisms (Manahan *et al.*, 1983; Davis and Stephens, 1984a, b; Lu Ming and Stephens, 1984). However, such work has often involved mixtures of amino acids which bear little resemblance to those found in the natural habitat of the organisms concerned. Since amino acids may interact during transport, the use of arbitrary mixtures as a basis for estimation of the total net entry from the environment is open to question.

In this paper we report net entry of each of eighteen amino acids from an equimolar mixture into the marine polychaete, *Pareurythoe californica*. A reduction in rate of entry for a representative of each of the major classes of amino acids is observed when rates are measured in the presence of an equimolar concentration of a representative of any of the other major classes (polar and nonpolar neutral, dicarboxylic, polybasic). Levels of free amino acids found in the natural habitat of the animals are then reported. Net entry is measured using mixtures of amino acids similar in composition and concentration to those found naturally. Finally, rates of net entry from these environmentally meaningful amino acid mixtures are compared with rates of nitrogen excretion and with measurements of aerobic metabolic rate to esti-

Received 15 October 1985; accepted 25 September 1986.

Abbreviations used: DOM (dissolved organic material), HPLC (high performance liquid chromatography), ASW (artificial seawater), OPA (ortho-phthaldialdehyde), DAPI (4,5-diamidino-2-phenylindole), AO (acridine orange).

mate the potential contribution of amino acid uptake to the metabolic requirements of the worms.

Materials and Methods

Animals

Pareurythoe californica was collected in the low intertidal area at San Onofre in southern California from the underside of cobbles which lie on a substrate of sand-gravel-rock. The worms were maintained in the laboratory at 23°C in an aquarium containing artificial seawater (ASW) prepared according to Cavanaugh (1964). The animals were fed once a week ("Seafood"; Instant Ocean Hatcheries). ASW was changed every second day. Animals were used within six weeks of collection.

Errant polychaetes such as *Pareurythoe* are opportunistic predators and scavengers, and under natural conditions they presumably feed frequently. As noted above, they were maintained in the laboratory by feeding once a week. Excess food was removed from the aquarium after each feeding. Under these conditions the worms may have starved periodically, which could have led to changes in rates of oxygen consumption, ammonia production, or amino acid uptake. Analysis of our data indicates no effect of our feeding regime on any of these parameters.

Environmental samples

Samples were taken of both the open water washing freely over the cobbles and of the water from the microhabitat of the worms under the cobbles. The latter samples were collected by inserting narrow-gauge tubing between and under undisturbed cobbles and drawing water slowly into a sterile 50 ml syringe. All water samples were filtered immediately in the field after collection, first to remove large particulates (0.45 μm; Millepore Type HA) and then to remove bacteria (0.2 μm; Nuclepore). The filtered samples were placed in sterile culture tubes and stored frozen for later analysis by HPLC.

The water samples were filtered under very gentle pressures. Neither the pressures generated nor the shear forces developed at the surface of the filter were great enough to damage any bacteria present. We have added bacteria to sterile ASW and filtered it, and see no disruption of the bacteria.

HPLC

HPLC methods similar to those employed by Jones and Gilligan (1983) were used to determine the levels of amino acids in the environmental samples, to measure rates of amino acid uptake and ammonia production in laboratory experiments, and to measure free amino acid pools in the organisms. Briefly, 800 μl of filtered sample, diluted if necessary with HPLC-grade distilled water, was combined with 200 μl of ortho-phthaldialdehyde (OPA) reagent and mixed vigorously. The resulting fluorescent amino acid derivatives were separated on a Beckman HPLC system. 500 μl aliquots of derivatized amino acid solution were injected onto an HPLC column assembly consisting of a guard column (70 mm long $\times$ 2.1 mm ID) packed with CO:PELL ODS (30 μm particle size; Whatman) followed by an Ultrasphere ODS analytical column (15 cm long $\times$ 4.6 mm ID; 5 μm particle size). Derivatives were eluted using gradient profiles composed of 0.05 *M* sodium acetate (pH 6.8), methanol, and small quantities of tetrahydrofuran as an organic modifier to improve separation of glycine and threonine. Peaks were detected and quantified using a Shimadzu C-E1B integrator. Actual concentrations of amino acids were determined by comparison with chro-

matographically pure standard mixtures. Standards were evaluated approximately every seventh run.

Uptake experiments

Net flux of amino acids was determined as follows. For each trial, one or two worms (about 70 mg wet wt) were washed repeatedly with sterile ASW. After washing, they were placed in a sterile beaker containing a solution of each of the amino acids to be tested at an initial concentration of 200 n*M* made up in 20 ml of autoclaved ASW. In each case a control group was set up without exogenous amino acid to monitor any possible leakage from the worms.

The beakers containing the worms were stirred at 50 rpm on an orbital shaker. This procedure provided moderate mixing without causing observable behavioral disturbances. At timed intervals, 2 ml samples were removed from the medium and immediately filtered through sterile filters (0.2 μm; Nuclepore) and frozen for subsequent analysis by HPLC. Rates of uptake were calculated based on the assumption of exponential depletion of amino acids from the medium. (This assumption was subsequently verified by least squares curve fitting on curves similar to those presented in Fig. 2.) The rate of uptake for each amino acid was calculated from the following expression:

$$K = (\ln [S_0] - \ln [S_t])/t$$

where K is the first order depletion coefficient, S_o is the substrate concentration at the start of the experiment, and S_t is the substrate concentration at time t. The depletion coefficient along with the volume of the medium and the concentration of each amino acid was used to calculate rates of uptake which are expressed as nmoles (gm wet wt)$^{-1}$ h^{-1}.

The rate of net exchange of individual amino acids, determined by HPLC, was compared with the rate of influx of the same ^{14}C-labeled amino acid in selected cases. These experiments were conducted as described above except that single amino acids were used and 0.5 μCi of uniformly labeled ^{14}C-amino acid was added to the initial incubation medium. At each sampling period, a 0.5 ml aliquot of the filtered sample was placed in a scintillation vial and acidified with 100 μl of 0.5 *N* HCl to volatilize any $^{14}CO_2$ formed during the course of the experiment. At least twelve hours after acidification, 4.5 ml of Aquasol II (New England Nuclear) was added to each vial. Twenty-four hours later the samples were counted on a Beckman CPM-100 scintillation counter.

Kinetics

The kinetics of influx were determined for three individual amino acids by determining the initial rates of uptake of ^{14}C-labeled amino acid from starting concentrations ranging from 300 n*M* to 500 μ*M*. Duplicate 0.5 ml samples were removed after 0, 3, 7, 10, and 15 minutes of incubation and prepared for scintillation counting as described above. Uptake rates were calculated by least squares regression of amino acid disappearance from the medium *versus* time. These rates were plotted *versus* their corresponding substrate concentrations and the kinetic parameters $J_{i(max)}$ (the maximal rate of influx) and K_t (concentration at which influx is half-maximal) were determined directly by non-linear least squares regression analysis (Duggleby, 1981).

Internal amino acid pools

To determine amino acid levels in the body wall of *Pareurythoe,* worms were washed in sterile ASW and a piece of body wall was dissected from the midsection.

The tissue was briefly rinsed in ASW, blotted, weighed on an analytical balance, dried to a constant weight at 90°C and reweighed. The dried tissue was rehydrated in HPLC-grade 80% ethanol and homogenized in a Dounce homogenizer. The homogenized tissue was allowed to extract for 24 hours at 4°C. The homogenate was then centrifuged at 2100 × *g* for 10 min. Aliquots of the supernatant were diluted with HPLC-grade distilled water and amino acid levels determined by HPLC.

Bacteria

Three separate procedures were used to evaluate the potential contribution of microorganisms to the observed uptake of amino acids by *Pareurythoe.*

(1) In conjunction with net flux experiments described above, a control group was set up to serve as a check for uptake due to epifauna loosely associated with the surface of the worms. Worms were placed on the orbital shaker in beakers of sterile ASW with no added substrate for 90 min. At this point, the animals were removed and sufficient amino acid stock solution was added to the medium to provide each of the 18 amino acids at a concentration of 200 n*M*. Disappearance of these added amino acids was followed by HPLC analysis of filtered medium samples taken over an additional 90-min period.

(2) Washed, intact animals were frozen with dry ice onto slides coated with gelatin-chrom alum. The frozen worms were embedded in Tissue-Tek (Miles Labs) at −27°C and 10 μm sections cut on a cryostat. The cut sections were placed on a cold slide, thaw-mounted and allowed to air-dry. The sections were stained for 1 min with either 4,5-diamidino-2-phenylindole (DAPI) at a final stain concentration of 0.01 μg ml^{-1}, or acridine orange (AO) at a final stain concentration of 10 μg ml^{-1}, then rinsed in McIlvaines buffer (pH 7.2). The sections were examined on a Zeiss standard microscope at 800× and 1000× with Neo-Fluor objectives. Epifluorescence was produced with a 100 W HBO mercury bulb and either a G 436 exciter filter with an FT 510 dichromatic beam splitter and an LP 515 Barrier filter (AO fluorescence) or a BP 365/10 exciter filter with an FT 390 chromatic beam splitter and an LP 395 barrier filter (DAPI fluorescence). As a check on the mounting and staining procedures, parallel samples of bacteria in detrital material collected from a lab aquarium were processed by the same procedures.

(3) Washed, intact animals were placed in a sterile culture tube and rinsed in a strong stream of ASW. The tube containing the animal was then vortexed for 90 s. The worm was removed and the ASW was treated by procedures similar to those of Daley and Hobbie (1975), Coleman (1980), and Porter and Feig (1980). The sample was fixed by addition of 6% formalin in ASW which had been freshly filtered (0.2 μm; Nuclepore). The sample was then filtered onto 0.2 μm Nuclepore filters which had been stained in Irgalan black (2 g l^{-1} plus 20 ml of acetic acid). The filters were rinsed with distilled water and counterstained with either DAPI or AO. The wet filters were mounted on slides coated with low fluorescence oil and coverslipped with oil. They were then examined by epifluorescence microscopy as described above. Parallel samples washed from detrital material were processed as controls.

Oxygen consumption

The oxygen consumption of *Pareurythoe* in the presence or absence of exogenous amino acids was measured using a Gilson differential respirometer. The reaction flask contained two animals in sterile ASW. After equilibration at 23°C for 30 min, sufficient amino acid stock solution to produce a 3 μ*M* solution or an equivalent volume

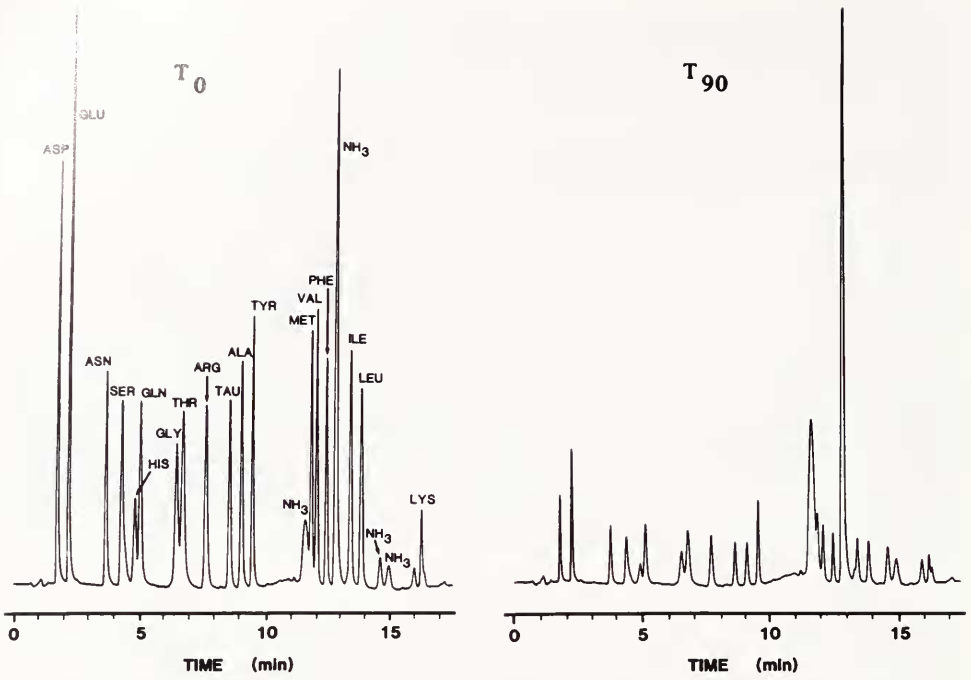

FIGURE 1. Net removal of 18 amino acids by *Pareurythoe*. The chromatogram at the left reflects the composition of the medium at time 0. The initial concentration of each amino acid in this medium was 200 n*M*. The chromatogram at the right shows the medium 90 min after addition of two worms (68 mg wet wt).

of sterile ASW was tipped into the reaction flask and oxygen consumption was measured for the next two hours.

RESULTS

The results shown in Figure 1 are typical of those obtained in six sets of observations measuring net entry of amino acids into *Pareurythoe* from the medium. The chromatogram labeled T_0 represents initial conditions where each of the 18 amino acids was present at a concentration of 200 n*M*. A significant proportion of all amino acids tested was removed during the 90 min incubation period as indicated in the chromatogram labeled T_{90}. This chromatogram also shows that ammonia was liberated over the course of the observations. Quantification of the areas of the peaks in these chromatograms yielded the average rates of uptake given in Table I. Analysis of variance showed that there was no significant difference in the rates of uptake among the polar neutral, nonpolar neutral, acidic, and basic amino acids tested ($P > 0.10$). The average total rate of uptake from our equimolar mixture (total concentration 3.6 μM) was 267.9 ± 18.5 nmoles (g wet weight)$^{-1}$ h^{-1}. The average ammonia production under these conditions was 547.6 ± 36.7 nmoles (g wet weight)$^{-1}$ h^{-1}.

Animals placed in sterile ASW without exogenous amino acids did not lose detectable amounts of any amino acid over a 90 min incubation period, but they did release ammonia at an average rate of 504.7 ± 43.1 nmoles (g wet weight)$^{-1}$ h^{-1}. This rate of ammonia release does not differ significantly from that of the experimental group above ($P > 0.10$). Given the volumes and tissue weights used in these experiments

TABLE I

Average rates of net uptake of 18 amino acids as determined by HPLC

Nonpolar neutral		Acidic	
ALA	16.87 ± 1.03	ASP	18.88 ± 0.71
ILE	15.64 ± 0.72	GLU	17.65 ± 0.73
LEU	15.49 ± 1.40		
MET	17.02 ± 1.60		
PHE	16.23 ± 1.01		
VAL	16.28 ± 0.56		
Polar neutral		**Basic**	
ASN	13.85 ± 0.74	ARG	14.80 ± 0.76
GLY	16.41 ± 1.24	HIS	12.42 ± 0.98
GLN	13.86 ± 1.88	LYS	10.79 ± 1.70
SER	16.20 ± 1.46		
THR	15.00 ± 1.31		
TYR	14.50 ± 0.71		

All values are expressed as nmoles (g wet wt)$^{-1}$ h^{-1} ± SEM. n = 6.

and the sensitivity of our HPLC detector, we could have easily quantified rates of amino acid loss as low as 1.2 nmoles (g wet weight)$^{-1}$ h^{-1}.

Entry of amino acids into *Pareurythoe* undoubtedly occurs at or via the external epithelium. Previous studies on adult marine annelids have shown that non-feeding animals do not drink (Ahearn and Gømme, 1975). It seems to be a general characteristic of uptake of amino acids in adult marine invertebrates that little or no entry occurs via the gut (Stephens, 1972).

To assess the potential contribution of epifaunal contaminants to the observed uptake of amino acids in the preceding experiments, we made one set of observations to measure the uptake by loosely attached epifauna and two separate sets of direct bacterial counts using epifluorescence microscopy. No epifaunal contamination was detected by these procedures although bacteria were clearly visible on the detrital samples processed as controls for the microscopy. Overall, our data support the view that *Pareurythoe* is the agent responsible for uptake, and that bacteria play a negligible role in the process.

Although we do not suggest that the surface of *Pareurythoe* is sterile, the low levels of bacteria that could have escaped our procedures could not provide a significant contribution to the amino acid uptake observed. These results are striking, but not wholly unexpected. Animals that produce surface mucus coats such as polychaete annelids, commonly secrete bacteriocidal or bacteriostatic compounds in the mucus (Rheinheimer, 1975). Also, scanning electron micrographs of *Nereis* prepared specifically to preserve epifauna show no visible contamination (Sieburth, 1965).

To estimate the concentration gradients against which the uptake of amino acids proceeds, amino acid levels in the body wall of *Pareurythoe* were determined by HPLC. The results of these analyses are presented in Table II. These concentrations represent minimum estimates of the intracellular amino acid concentrations in the transporting epithelium since they are based on the total water content of the tissue rather than its intracellular water content. Gradients (internal:external) calculated on the basis of these internal amino acids levels and the initial concentrations present in the test mixture of 18 amino acids ranged from 2×10^4 for histidine to 5×10^5 for asparagine. These are minimum values both because of the probable underestimate of actual intracellular concentrations noted above and because all of the amino acids

TABLE II

Concentrations of amino acids in the body wall of Pareurythoe

Amino acid	Conc. (m*M*)	Amino acid	Conc. (m*M*)
ASN	102.41	ARG	14.05
ALA	31.58	VAL	13.56
ASP	31.02	ILE	13.42
GLU	27.37	GLN	13.38
LYS	19.59	THR	10.78
LEU	18.95	PHE	9.68
MET	17.55	TYR	9.04
SER	15.41	TAU	5.63
GLY	14.86	HIS	4.37

Total amino acid concentration = 372.66 m*M*.
n = 3.

supplied in the medium were reduced substantially below their initial concentration of 200 n*M* during the observations.

To clarify the relationship between influx and the net entry of individual amino acids, simultaneous measurements of influx (disappearance of ^{14}C-labeled amino acid from the medium) and net flux (decrease in concentration of the same amino acid as measured by HPLC) were performed. Such measurements were made for representative acidic (aspartate), basic (lysine), nonpolar neutral (alanine, glutamine), and polar neutral amino acids (serine, glycine). The initial concentration in the incubation medium (^{14}C-labeled plus unlabeled) ranged from 300 to 400 n*M* depending on the specific activity of labeled compounds. Fluxes were measured for 90 min. The results shown in Figure 2 for serine are typical. In no case did least squares regression analysis of the influx and net flux data yield curves whose coefficients were significantly different.

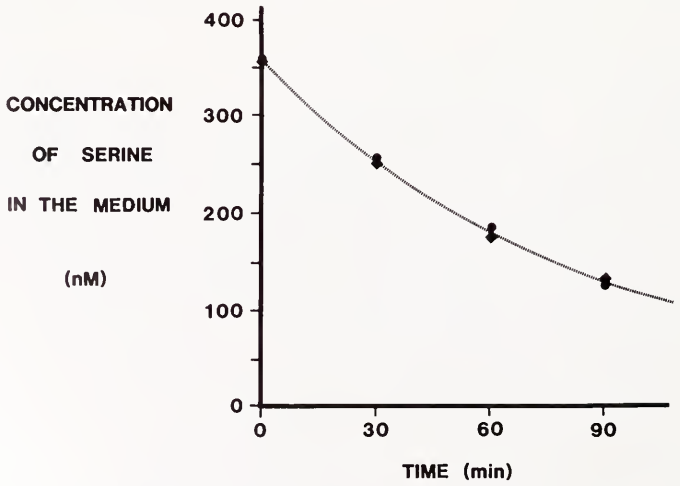

FIGURE 2. Influx of labeled serine compared to net serine flux as measured by HPLC. Influx data are represented by diamonds (♦). Net flux data are represented by circles (●). The exponential curve was fit to both data sets by least squares regression ($r^2 = 0.9975$).

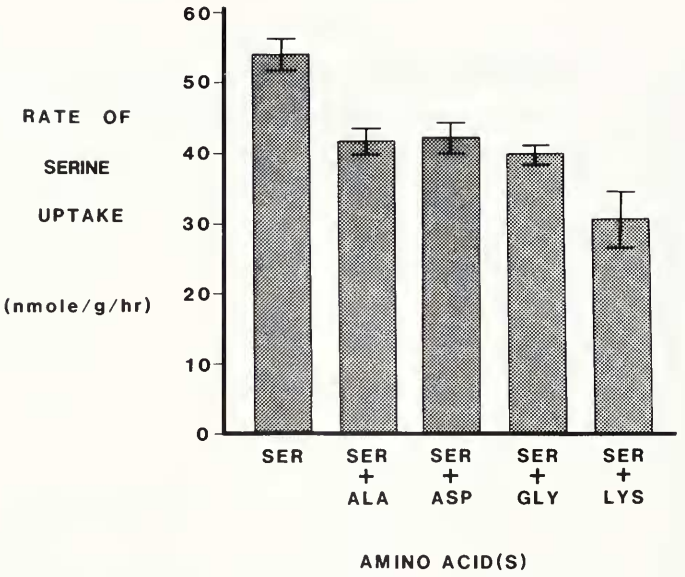

FIGURE 3. Inhibition of serine uptake by equimolar amounts of amino acids from each of the major amino acid classes. Each amino acid was present in the medium at an initial concentration of 200 n*M*. Net flux of each of the amino acids was determined by HPLC analysis of samples of the medium.

The kinetics of influx were investigated for serine, glycine, and alanine. The relationships between the rates of influx for each of these amino acids and their concentrations in the external medium were well described by the Michaelis-Menten equation. Values for $J_{i(max)}$ and K_t were determined directly from Michaelis curves by nonlinear least squares regression analysis. $J_{i(max)}$ values obtained were 3.6 (serine), 4.3 (glycine), and 6.1 (alanine) expressed as μmoles (g wet weight)$^{-1}$ h^{-1}. The corresponding values for K_t (μM) were 29.5 (serine), 36.9 (glycine), and 66.3 (alanine).

It was noted in the above observations that entry rates for these amino acids when presented singly were higher than those in experiments using our test mixture of 18 amino acids. We therefore examined possible interactions between amino acids. Uptake of representatives from each major class was measured when each amino acid was presented alone and when it was paired with an equimolar amount (200 n*M* each) of an amino acid from each class. An exhaustive set of pairings was constructed using serine, glycine, alanine, aspartate, and lysine as substrates. All of the amino acid pairings tested resulted in a 20–40% inhibition of uptake compared to the rate observed when the amino acid was presented alone. The results presented for serine (Fig. 3) are typical.

HPLC analysis of water samples taken at the collection site at San Onofre produced data summarized in Table III. Total amino acids present in the water washing freely over the cobbles ranged from 115 to 350 n*M;* from 8 to 17 different amino acids were identified in the nine samples analyzed. Each of the five water samples taken from under the cobbles contained all 18 amino acids we measured with total concentrations ranging from 3.6 to 17.5 μM. Table III presents the mean value of samples measured. The microhabitat of *Pareurythoe* was richer in both diversity and concentration than the surrounding open seawater. Six amino acids were consistently more abundant in the samples with serine, glycine, alanine, glutamate, aspartate, and lysine accounting for more than 80% of the total present.

TABLE III

Average amino acid concentrations at San Onofre

Amino acid	Microhabitat water	Open water
ASP	652 n*M*	16 n*M*
GLU	261	11
ASN	118	3
SER	3129	39
HIS	120	8
GLN	685	7
GLY	2062	36
THR	533	6
ARG	96	—
TAU	57	—
ALA	944	16
TYR	208	—
MET	134	7
VAL	276	5
PHE	144	—
ILE	261	—
LEU	232	—

Microhabitat water n = 5; open water n = 9.

As noted above, interactions among amino acids affected their rates of influx. Therefore, rates of uptake from an amino acid mixture simulating observed substrate composition in the natural habitat were determined and used to make more realistic estimates of entry under natural conditions. The mixture employed contained the six most abundant amino acids from the microhabitat samples present in proportion to their abundance in these samples. The total amino acid concentration was 3.0 μM (*i.e.*, slightly less than the minimum total concentration measured in the microhabitat of the worms at the collection site). Average rates of amino acid uptake from this mixture are listed in Table IV.

The aerobic metabolic rate of *Pareurythoe* was measured in ASW containing either serine, glycine, alanine (at a concentration of 7 μM) or without added substrate. Analysis of variance showed the presence of exogenous amino acids caused no significant change in oxygen consumption. The overall average oxygen consumption was 198 ± 13 μl (g wet weight)$^{-1}$ h^{-1}. Based on the rates of uptake of the six major constituents listed in Table IV, this corresponds to the oxidation of approximately 3.2 μmoles of these amino acids.

DISCUSSION

We have used HPLC analysis to demonstrate net entry of each of an equimolar mixture of 18 amino acids each supplied at an initial concentration of 200 n*M* (total concentration 3.6 μM). For six individual amino acids chosen to represent the four major classes of amino acids, influx as measured by the disappearance of ^{14}C-labeled substrate equaled rate of net entry as measured by direct chemical quantification via HPLC. These observations exclude the possibility of accounting for the observed influx of labeled amino acids by postulating exchange diffusion (Johannes *et al.*, 1969).

Animals incubated for 90 min in ASW did not lose detectable amounts of any amino acid though they did continue to produce ammonia at rates comparable to those shown by animals exposed to the test amino acid mixture. This observation is

TABLE IV

Amino acid composition of artificial seawater solutions designed to mimic the natural microhabitat of Pareurythoe *and rates of uptake from these solutions*

Amino acid	Concentration (n*M*)	Rates of amino acid uptake [nmoles (g wet wt)$^{-1}$ h^{-1}]
SER	1050	123.49 + 2.67
GLY	750	97.89 + 5.74
ALA	300	37.57 + 0.67
ASP	300	32.19 + 0.26
GLN	300	39.29 + 0.29
LYS	300	17.96 + 5.23
Total	3000	348.39 + 2.48

n = 5.

in agreement with the bulk of observations in the literature. Leakage of amino acids from marine invertebrates can be induced by previous exposure of the animals to high concentrations of substrate (*e.g.,* Jorgensen, 1980; Davis and Stephens, 1984a), but has not been convincingly demonstrated except in cases involving special experimental procedures. Transepidermal leakage has been postulated by Gømme (1982) and by Wright and Secomb (1984). However, this proposed leakage is accompanied by localized active resorption such that net loss to the bulk medium is not measurable.

Rates of uptake by *Pareurythoe* are comparable for all 18 of the amino acids examined. This is unusual among annelids which have been studied. Stephens (1975) reported that *Nereis diversicolor* takes up neutral amino acids more rapidly than acidic amino acids and Jorgensen (1979) reported a similar situation for *N. virens.* Other marine invertebrates vary in the extent to which they discriminate among classes of amino acids. The bivalve, *Mytilus edulis,* exhibits rather catholic tastes (Manahan *et al.,* 1982) while echinoderm larvae strongly prefer neutral amino acids (Davis and Stephens, 1984a, b).

The rate of uptake of amino acids by *Pareurythoe* is relatively rapid compared to that of other animals collected from the same microhabitat at San Onofre (*i.e.,* under the cobbles). Uptake from the test solution of 18 amino acids by *Pareurythoe* was 268 nmoles (g wet weight)$^{-1}$ h^{-1}. Under identical conditions, the apodus sea cucumber, *Leptosynapta albicans,* removed 16 of the 18 amino acids. The total uptake was 97 nmoles (g wet weight)$^{-1}$ h^{-1}, neutral amino acids were removed much more rapidly than acidic amino acids and basic amino acids were not taken up at measurable rates. We were unable to demonstrate net entry of any amino acids in the flatworm, *Notoplana acticola.* In previous work, Davis *et al.* (1985) examined uptake of 15 amino acids, each at an initial concentration of 125 n*M,* by the brittle star *Ophionereis annulata* collected at the same location. Recalculating from their data to the higher concentration used in the present study produces a rate of 67 nmoles (g wet weight)$^{-1}$ h^{-1}.

The kinetic parameters reported here for *Pareurythoe* fall well within the range of those reported for epidermal amino acid transport for other marine annelids (see Stephens, 1972; Wright and Stephens, 1978). However, kinetic data such as these should be interpreted cautiously. The presence of unstirred layers at epithelial surfaces is now realized to have important consequences for the interpretation of kinetic data for transport in vertebrate intestine (Winne, 1973; Thomson and Dietschy, 1980). This has been shown to apply to transport by invertebrate epithelia as well (Wright *et al.,* 1980). The fact that the epidermal surfaces of marine polychaetes show a significant unstirred layer is understandable in terms of their architecture. In addi-

tion to the surface mucus coat, the epidermis of annelids has a protein-polysaccharide cuticle. Gømme (1982) presented evidence that the cuticle reduces the mobility of small molecules by two to three orders of magnitude compared to the bulk aqueous medium. This would enhance the discrepancy between observed bulk kinetic parameters and the actual behavior of the transporters in the epithelium.

As reported in Results, the uptake of representatives of all of the major classes of amino acids was influenced by the presence of equimolar amounts of other amino acids, even at very low concentrations. We have not attempted to explore the details of kinetics of such interactions. However, the results are reported here because they are obviously germane to estimation of net influx under natural conditions.

Water samples taken from the site at which *Pareurythoe* was collected indicate that the microhabitat of the worms was rich in free amino acids compared to the open water washing over the cobbles under which they are found. Total concentration in the microhabitat was roughly 10 μM while the open water averaged about 100 nM. This range of concentrations agrees with previous studies of amino acid levels in comparable environments (Stephens, 1972; Crowe *et al.*, 1977; Henrichs and Farrington, 1979; Davis *et al.*, 1985). The high degree of spatial and temporal variability in levels of amino acids in both habitats make the ranges rather broad.

Estimates of the potential contribution of exogenous amino acids to the nutritional and metabolic requirements of *Pareurythoe* were made by correlating rates of uptake from mixtures consisting of the six amino acids most prominent in the habitat of the worms with rates of oxygen consumption. The mixture employed contained the amino acids in proportion to their observed natural abundance with a total concentration of 3 μM, *i.e.*, slightly below the minimum total concentration measured in the field. At this concentration, amino acid uptake could account for roughly 10% of the reduced carbon required to sustain oxidative metabolism. Extrapolation to the maximum total concentration measured in samples from the microhabitat (about 18 μM) suggests that as much as 50% of the carbon required for oxidative metabolism could be supplied by uptake.

These figures are based on comparison with the measured rate of oxygen consumption and make the further assumption that the amino acids acquired by this pathway are fully oxidized. They are not intended to be interpreted as statements concerning the actual fate of exogenous amino acids acquired from the environment. Neither are they intended to defend the position that the metabolism of these worms is exclusively aerobic in character. With respect to the fate of exogenous amino acids, we know from numerous studies in the literature as well as observations on *Pareurythoe* not cited here that they are incorporated into various compounds in the organisms as well as persisting in the internal free amino acid pool for considerable periods of time. The comparison is designed to call attention to the fact that the input of external amino acids via this pathway presumably spares provision of reduced carbon by other pathways which would be required to sustain carbon balance and provide for growth in the absence of such uptake from the environment.

Similarly, the comparison of the amino nitrogen provided by amino acid uptake with the rate of ammonia excretion is not intended to imply that the amino acids are promptly deaminated on entry and constitute the proximate source of ammonia. Again, it is drawn to indicate that entry of amino nitrogen by this pathway spares other potential nutritional sources and/or utilization of internal reserves present in the animals.

ACKNOWLEDGMENTS

We would like to thank Catherine Ulibarri of the Department of Psychobiology, UC Irvine, for the use of and assistance with the fluorescence microscope.

This work was supported by National Science Foundation Grant PCM 82-08185.

LITERATURE CITED

AHEARN, G. A., AND J. GØMME. 1975. Transport of exogenous D-glucose by the integument of a polychaete worm (*Nereis diversicolor* Muller). *J. Exp. Biol.* **52:** 243–264.

CAVANAUGH, G. M. 1964. *Formulae and Methods, V, of the Marine Biological Laboratory Chemical Room.* Marine Biological Laboratory, Woods Hole, MA.

COLEMAN, A. W. 1980. Enhanced detection of bacteria in natural environments by fluorochrome staining of DNA. *Limnol. Oceanogr.* **25:** 948–951.

CROWE, J. H., K. A. DICKSON, J. L. OTTO, R. D. COLON, AND K. K. FARLEY. 1977. Uptake of amino acids by the mussel *Modiolus demissus. J. Exp. Zool.* **202:** 323–332.

DALEY, R. J., AND J. E. HOBBIE. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. *Limnol. Oceanogr.* **20:** 875–882.

DAVIS, J. P., AND G. C. STEPHENS. 1984a. Uptake of free amino acids by bacteria-free larvae of the sand dollar *Dendraster excentricus. Am. J. Physiol.* **247:** R733–R739.

DAVIS, J. P., AND G. C. STEPHENS. 1984b. Regulation of net amino acid exchange in sea urchin larvae. *Am. J. Physiol.* **247:** R1029–R1037.

DAVIS, J. P., G. C. STEPHENS, AND M. A. RICE. 1985. Net entry of amino acids into the brittle star, *Ophionereis annulata. Comp. Biochem. Physiol.* **81A:** 899–903.

DUGGLEBY, R. G. 1981. A nonlinear regression program for small computers. *Anal. Biochem.* **110:** 9–18.

GØMME, J. 1982. Epidermal nutrient absorption in marine invertebrates: a comparative analysis. *Am. Zool.* **22:** 691–708.

HENRICHS, S. M., AND J. W. FARRINGTON. 1979. Amino acids in interstitial waters of marine sediments. *Nature* **279:** 319–322.

JOHANNES, R. E., S. J. COWARD, AND K. L. WEBB. 1969. Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.* **29:** 283–288.

JONES, B. N., AND J. P. GILLIGAN. 1983. O-phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J. Chromatogr.* **266:** 471–482.

JORGENSEN, N. O. 1979. Uptake of L-valine by the polychaete *Nereis virens. Mar. Biol.* **52:** 45–52.

JORGENSEN, N. O. 1980. Uptake of glycine and release of primary amines by the polychaete *Nereis virens* (Sars) and the mud snail *Hydrobia neglecta* (Muus). *J. Exp. Mar. Biol. Ecol.* **47:** 281–297.

LU MING, AND G. C. STEPHENS. 1984. Demonstration of net influx of free amino acids *Phaeodactylum tricornutum* using high performance liquid chromatography. *J. Phycol.* **20:** 584–589.

MANAHAN, D. T., J. P. DAVIS, AND G. C. STEPHENS. 1983. Bacteria-free sea urchin larvae take up neutral amino acids selectively from seawater. *Science* **220:** 204–206.

MANAHAN, D. T., S. H. WRIGHT, G. C. STEPHENS, AND M. A. RICE. 1982. Transport of dissolved amino acids by the mussel *Mytilus edulis:* demonstration of net uptake from natural seawater. *Science* **215:** 1253–1255.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25:** 943–948.

RHEINHEIMER, G. 1975. *Mikrobiologie der Gewasser.* Gustav Fischer Verlag, Stuttgart.

SIEBURTH, J. M. 1965. *Microbial Seascapes.* University Park Press, Baltimore, MD.

STEPHENS, G. C. 1972. Amino acid accumulation and assimilation in marine organisms. Pp. 155–184 in *Nitrogen Metabolism and the Environment,* J. W. Campbell and L. Goldstein, eds. Academic Press, New York.

STEPHENS, G. C. 1975. Uptake of naturally occurring primary amines by marine annelids. *Biol. Bull.* **149:** 397–407.

THOMSON, A. B., AND J. M. DIETSCHY. 1980. Experimental demonstration of the effect of the unstirred water layer on the kinetic constants of the membrane transport of D-glucose in rabbit jejunum. *J. Membr. Biol.* **54:** 221–229.

WINNE, D. 1973. Unstirred layer, source of biased Michaelis constant in membrane transport. *Biochim. Biophys. Acta.* **298:** 27–31.

WRIGHT, S. H., AND T. W. SECOMB. 1984. Epidermal taurine transport in marine mussels. *Am. J. Physiol.* **247:** R346–R355.

WRIGHT, S. H., S. A. BECKER, AND G. C. STEPHENS. 1980. Influence of temperature and unstirred layers on the kinetics of glycine transport in isolated gills of *Mytilus californianus. J. Exp. Zool.* **214:** 27–35.

WRIGHT, S. H., AND G. C. STEPHENS. 1978. Removal of amino acid during a single passage of water across the gill of marine mussels. *J. Exp. Zool.* **205:** 337–351.